remain a frustrating unknown until considerably more research is done.

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Sexual Dimorphism: Can You Smell the Difference?

A powerful new technique for visualizing neurons in the fly brain has uncovered fine neuroanatomical differences between the olfactory circuitries of male and female *Drosophila*.

Elizabeth J. Rideout and Stephen F. Goodwin*

Dramatic behavioral differences exist between males and females in *Drosophila* [1–3]. Although the genetic basis for the creation of two morphologically and behaviorally distinct sexes has been extensively studied, few anatomical differences in the brain have been identified which may explain these dimorphic behaviors [4–7]. In fact, gross anatomical studies suggest that male and female brains are largely similar [5]. Identifying potential differences requires detailed analyses of the neural circuits underlying these sex-specific behaviors. Currently, identifying and tracing the projections of single neurons or populations of neurons relies upon clonal analysis techniques, which can be time-consuming due to the stochastic nature of generating uniquely labelled neurons [8,9].

Now, Datta *et al.* [10] have described a novel system for visualizing the cell bodies and projections of single neurons with high spatial and temporal resolution, using a photoactivatable green fluorescent protein (PA-GFP) [11]. PA-GFP is a stable photoactivatable variant of GFP that after irradiation with 413 nm light shows a 100-fold increase in fluorescence when excited by 488 nm light [11]. Significantly, when PA-GFP is photoactivated in an isolated region of the cell, it will then diffuse throughout the entire cell. Datta *et al.* [10] exploited this property of PA-GFP to locate cell bodies of neurons, and to visualize axonal projections of identified neurons. Using this elegant technique, the authors were able to visualize and identify sexually dimorphic axonal projections of a specific population of neurons in the brain.

During courtship, male and female flies exchange a variety of stimulatory and inhibitory sensory cues [12]. Intriguingly, certain auditory and olfactory cues elicit opposite behavioral responses from the two sexes [13,14]; for example, *cis*-vaccenyl acetate, a male-specific pheromone, has been shown to have an inhibitory effect on male courtship, whereas in females, *cis*-vaccenyl acetate enhances receptivity to copulation [14]. How can a single pheromone elicit such different behavioral responses? Datta *et al.* [10] addressed this question by investigating whether anatomical and/or functional differences in olfactory neural circuitry may explain this dimorphic behavior (Figure 1).

In flies, the physiological response to *cis*-vaccenyl acetate is mediated by a class of olfactory sensory neurons expressing the odorant receptor gene *Or67d*. All neurons expressing *Or67d* innervate a single glomerulus, DA1, in the antennal lobe [14]. Although the size of the DA1 glomerulus is sexually dimorphic [5,14,15], Datta *et al.* [10] found no essential differences between males and females in either the increase of intracellular Ca^{2+} concentration or in the electrophysiological responses in the DA1 glomerulus following exposure to *cis*-vaccenyl acetate, suggesting that the neurobiological basis for the sex-specific responses to *cis*-vaccenyl acetate must lie elsewhere in the olfactory neural circuit.

Next, using PA-GFP, they determined that projection neurons which project from the DA1 glomerulus to the lateral horn, a higher olfactory processing centre, have sexually dimorphic axonal arbors on the lateral horn (Figure 1). After identifying these sexually dimorphic arbors, Datta *et al.* [10] went on to show that this dimorphism depends on the expression of the male-specific isoform

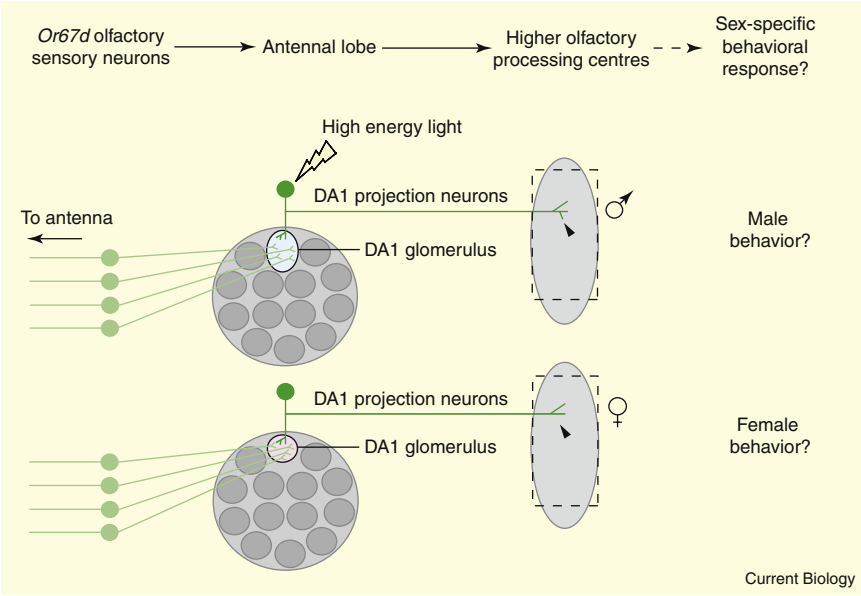

Figure 1. Sexual dimorphism in higher olfactory processing centres.

The *Drosophila* olfactory circuitry. An odor is detected by an olfactory sensory neuron expressing a single type of odorant receptor gene (*Or*). All olfactory sensory neurons expressing a particular *Or* project their axons to a single glomerulus in the antennal lobe, where they form synapses with projection neurons, which transmit olfactory information to higher olfactory processing centres: the mushroom bodies and the lateral horn. Olfactory sensory neurons expressing *Or67d* largely govern the fly's physiological response to the male-specific pheromone *cis*-vaccenyl acetate [14], and project their axons to the DA1 glomerulus in the antennal lobe. DA1 is sexually dimorphic in volume, but no dimorphisms in the electrophysiological response to *cis*-vaccenyl acetate were detected in this glomerulus. Using photoactivatable green fluorescent protein (PA-GFP) to visualize both individual DA1 projection neurons, and subpopulations of DA1 projection neurons, Datta *et al.* [10] investigated whether a dimorphism occurs in the transmission of olfactory information to higher olfactory processing centres. A dimorphic axonal arbor of DA1 projection neurons on the lateral horn was uncovered (black arrowheads), where males, projection neurons had additional axonal branches. However, the impact of this dimorphism on behavior remains unknown (dashed line).

of *fruitless* (*fru*), a sex determination gene required for the performance of many male behaviors [1]. Previously, the dimorphism in DA1 glomerular volume had been shown to depend on *fru* [5]. These new findings extend our understanding of *fru*'s role in shaping sex-specific olfactory circuitry [10]. In the future, it will be interesting to see whether *fru*'s male-specific isoforms (Fru^M) are also responsible for the creation of other dimorphisms in olfactory circuitry, such as those recently reported by Jefferis *et al.* [16] in the lateral horn.

Although Datta *et al.* [10] were not able unequivocally to link these dimorphisms in DA1 projection neurons to specific behavioral outcomes, they have developed a strategy for targeted neural tracing that allows electrophysiological recordings to be taken from identified neurons. This approach will undoubtedly be exploited to identify additional dimorphic circuitry in the *Drosophila* brain. It is important to mention, however, that in trying to catalogue sexual dimorphism in the brain, there are examples where equivalent neurons are not present in both sexes for comparison purposes [4–7]. In fact, one of these studies [4] showed that, for a cluster of neurons dorsal to the antennal lobe, both the presence of the neuron and its axonal morphology depend on the presence of Fru^M proteins. Together with the findings from Datta *et al.* [10], these results suggest that *fru*'s influence on male courtship behavior is a product of regulating dimorphism in the brain at many levels. Along with other sex determination genes, *fru* specifies dimorphic neuronal populations, and in addition, dimorphic axonal and dendritic projections, factors which all contribute to sex-specific behaviors.

So how does the identification of anatomical differences between males and females allow us to gain the necessary insights into the origins of sexually dimorphic behaviors? First, the structure of the *Drosophila* brain has been well studied, and various regions of the brain are known to be associated with specific behaviors; for example, the ability to learn and remember is intimately linked with the mushroom bodies. Therefore, by understanding where the dimorphisms between males and females lie, the significance of any existing dimorphisms can be inferred, and later tested. Second, as Datta *et al.* [10] indicate, the ability to label specific neurons or specific subsets of neurons in the living brain will allow recordings to be taken from these identified neurons in flies (or indeed other animals) as they are exposed to specific cues, or even as they are behaving, allowing the function of a dimorphic neuron to be related to behavior.

Finally, experience-dependent changes in specific brain structures such as the mushroom bodies have been reported [17]; in the future, it will be interesting to see if this new system for visualizing specific structures and/or neurons can be used to investigate the dynamics of these experience-dependent changes in the brain, giving vital insights into the mechanisms underlying both synaptic and behavioral plasticity. Therefore, this elegant new technique for visualizing and recording from specific neurons moves us another step closer to understanding the relationship between the development and function of the brain, and behavior.

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Visual Categorization: When Categories Fall to Pieces

We cannot help but categorize the visual world into objects like cats and faces. An intriguing new study shows that observers automatically discover informative fragments of visual objects during category learning.

Quoc C. Vuong

We see the world in discrete categories in order to recognize and interact appropriately with objects in our environment [1]. How do we learn visual object categories? Our intuition suggests that, through experience, we acquire features found in members of one category but not in those from another category. For example, cats have whiskers; human faces, on the other hand, normally do not. There is empirical support for this intuitive view [2,3].

But a fundamental problem with this intuition is image variability. Familiar objects from the same category can have an enormous range of appearance; they are often occluded by other objects; how they appear to us can further be confounded by viewing conditions such as variable illumination; and so on [2]. These factors converge to make it extremely difficult to learn generic features that are reliable for visual categorization.

In work published recently in *Current Biology*, Hegdé *et al.* [4] offer a compelling solution to this problem, but one that highlights the need for us to re-think the pieces that make up objects and object categories. Armed with a set of novel visual categories [5] and a statistical means to select features [6,7], these authors have demonstrated that observers automatically discover fragments — literally, bits and pieces of images — during category learning that are very effective for visual categorization. This provides a new and important link between visual category learning and visual categorization.

In this new study [4], observers classified a large number of unfamiliar objects into two categories. The objects were synthesized from a novel virtual phylogenesis algorithm which simulated the evolution of biological forms [5], so that category members captured natural variations of categories we are more familiar with. The examples in Figure 1 show that this classification task is far from trivial, even with whole objects (see supplemental Figure S1 in the paper for more examples).

Two main sets of image fragments were extracted from trained objects using the same statistical procedure. Observers then classified all fragments, just as they had done with whole objects. This sounds like an even more daunting task. Amazingly though, observers were as accurate with one set of fragments as they were

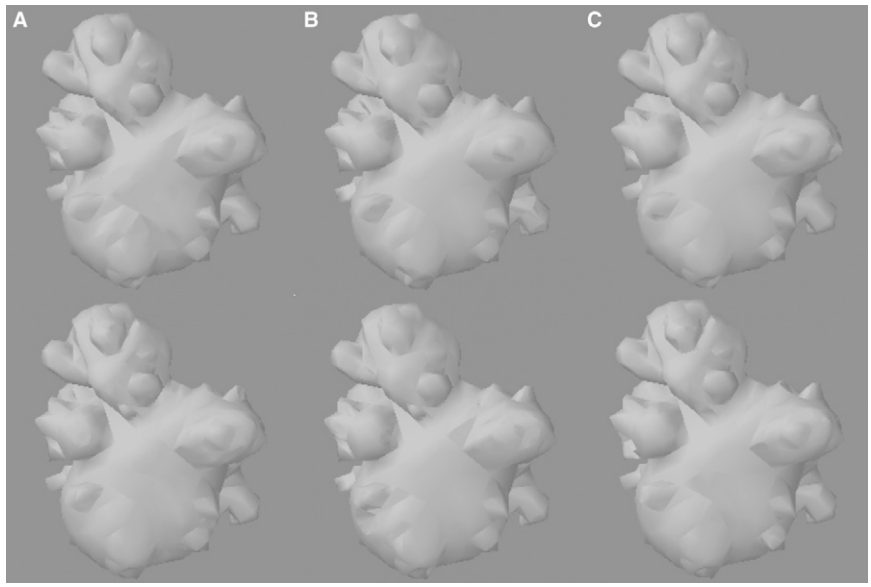

Figure 1. Example objects synthesized by virtual phylogenesis.
Observers were only trained on objects from two of the three categories A, B and C (from [4]).